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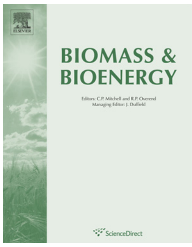

Hydrothermal liquefaction (HTL) of microalgae for biofuel production: State of the art review and future prospects

Diego López Barreiro [a,*], *Wolter Prins* [a], *Frederik Ronsse* [a], *Wim Brilman* [b]

[a] *Department of Biosystems Engineering, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B9000 Ghent, Belgium*
[b] *Thermo-Chemical Conversion of Biomass Group, Faculty of Science and Technology, University of Twente, P.O. Box 217, 7500AE Enschede, The Netherlands*

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ABSTRACT

Among the various types of biomass, microalgae have the potential of becoming a significant energy source for biofuel production in the coming years. Currently, research is mainly focusing on optimization of the cultivation methods and the conversion of just a single microalgae fraction (lipids for biodiesel production). Hydrothermal liquefaction is a method for thermochemical conversion of wet microalgae, producing a liquid energy carrier called 'bio-oil' or 'biocrude', next to gaseous, aqueous and solid by-products. A review of the available literature is presented here, analyzing the influence of parameters such as temperature, holding time and catalyst dosage on the yield and properties of the different product fractions. Also, the strain selection and the status of the technology for hydrothermal processes are analyzed. Finally, based on the findings obtained from the literature review, directions for future research are suggested.

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1. Introduction

Biomass can be converted by several processes to intermediate solid, liquid or gaseous biofuels that are said to have the potential of supplying 30% of the global fuel demand without affecting the food production and being respectful to the environment [1]. Microalgae are microscopic photosynthetic organisms that can be considered as versatile biological cell factories for renewable biofuel production, with higher photosynthetic efficiency, faster growth rates and higher area-specific yields than terrestrial biomass. They have simple growth needs (water, light, a carbon source and nutrients), and their main constituents are proteins, carbohydrates and lipids. Another fraction, the algaenans, has gained recently attention as source of biofuels [2,3]. These algaenans are insoluble non-hydrolysable biomacromolecules, resistant to drastic chemical treatments, in the form of long aliphatic chains that can be found in the outer wall of the microalgae cells [4], mainly from freshwater strains [5].

Cultivation of microalgae brings along some advantages [6,7]: they can be used in waste water treatment while using NH_4^+, $N0_3^-$ or PO_4^{3-} from the aqueous waste streams as nutrient sources; they can be grown in areas unsuitable for agriculture uses, thus not competing with food production; they can be used for the production of valuable co-products (such as proteins) and they can reduce CO_2 emissions from industrial

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* *Corresponding author*. Tel.: +32 (0)9 264 61 90; fax: +32 (0)9 264 62 35.
E-mail address: Diego.LopezBarreiro@UGent.be (D. López Barreiro).

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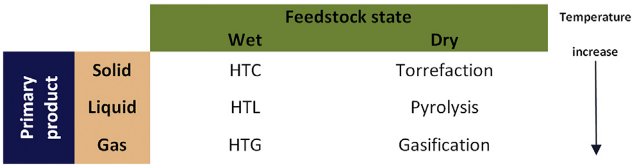
processes due to their high capacity of CO_2 fixation. Also, some drawbacks need to be mentioned: cultivation and harvesting are still too expensive; most microalgal metabolic pathways are still not fully understood; the low cell densities required to enhance light energy fixation demand high surface areas for the cultivation units and the input cost for nutrients (when not available from waste water) is very high.

During the past decades, microalgal research has been focused mainly on optimization of cultivation methods, but less effort was spent to their processing for biofuels, chemicals or energy production. Due to their high mass fraction of water (80–90%) [8], traditional thermochemical processes like pyrolysis and gasification are economically not very interesting [9]. Thermochemical processes for wet biomass, such as hydrothermal gasification, hydrothermal liquefaction or hydrothermal carbonization appear to be more suitable for microalgae feedstock. Hydrothermal liquefaction (HTL) is an approach that requires no drying because the whole microalgae biomass is decomposed and converted in hot compressed water. A biocrude oil is obtained as the main product, next to gaseous, aqueous and solid by-products. Upon combustion, this biocrude oil has low sulfur emissions and its low ash content results in reduced emissions of particulates, although it may lead to high NO_x emissions, due to the high amounts of nitrogen in chlorophyll and proteins [10], very abundant in microalgae cells. This last issue is one of the most challenging issues that HTL will need to overcome in order to become a real alternative for biofuel production from microalgae.

HTL has undergone in recent years a sudden increase in the number of publications. However, the knowledge generated is rather fragmentary, and therefore the intention of this review is to bring together and analyze the available data. Comparing the research findings appeared to be quite challenging due to the wide variation in the microalgae strains used, their initial state, the reaction conditions or the catalysts applied.

2. Thermochemical conversion schemes for microalgae biofuels production

The main routes for biofuel production from microalgal feedstock can be divided into chemical, biochemical and thermochemical. The kind of biofuel obtained and its mass fraction from the original feedstock is directly influenced by the process conditions. For the purpose of this paper, the term yield will refer to the mass fraction of the dry input biomass and will be expressed as percentage. For thermochemical conversion, process conditions are strongly affected by the high content of water from microalgal feedstock. Fig. 1 represents the various thermochemical conversion technologies for biofuel production from microalgae, classifying them according to the desired primary product and the water content of the feedstock.



Primary product		Feedstock state: Wet	Feedstock state: Dry
	Solid	HTC	Torrefaction
	Liquid	HTL	Pyrolysis
	Gas	HTG	Gasification

Fig. 1 – Thermochemical conversion processes for microalgal feedstock (HTC = hydrothermal carbonization; HTL = hydrothermal liquefaction; HTG = hydrothermal gasification).

2.1. *Dry processes*

2.1.1. *Torrefaction*

Torrefaction is a thermochemical conversion technique that processes fairly dry biomass at mild conditions (200–300 °C and at atmospheric pressure), under the absence of oxygen, causing a partial decomposition of the biomass and yielding a charry solid material as main product. To the best of our knowledge, no study is available for microalgae torrefaction. The high energy costs associated with the use of dry microalgae, together with the low value of the product obtained, clearly discourages research on this field.

2.1.2. *Pyrolysis*

Pyrolysis is the thermal treatment of biomass in the absence of oxygen, converting dry biomass into bio-oil, charcoal and a gaseous fraction at temperatures of 400–600 °C and atmospheric pressure. It is usually divided into fast and slow pyrolysis. Fast pyrolysis requires high biomass particle heating rates (e.g. 1000 °C min^{-1}) and short vapor residence times (in the order of seconds), thus producing directly a liquid fuel. Slow pyrolysis uses lower biomass particle heating rates (5–80 °C min^{-1}) and longer vapor residence times (5–30 min) [11], enhancing the production of tars and char.

For slow pyrolysis, several studies have investigated the behavior of *Chlorella protothecoides* and *Spirulina platensis* [12,13], obtaining reduced bio-oil yields. The experimental conditions vary from temperatures from 200 to 800 °C, heating rates from 15 to 80 °C min^{-1} and residence times as long as 100 h. This process led to the production of gases mainly composed of CO_2 and CH_4. The influence of temperature (200–600 °C) and holding time (5–120 min) was studied by Peng et al. [14], who found that the gas yield was generally higher when increasing the temperature and holding time.

With regard to fast pyrolysis, Miao et al. [15] tested *Chlorella protothecoides* and *Microcystis aeruginosa* at 500 °C, with a heating rate of 600 °C min^{-1} and a vapor residence time of 2–3 s, obtaining bio-oil yields of 18 and 24%, respectively. In another paper [16], *Chlorella protothecoides* was tested under the same heating rate and vapor residence time mentioned before, but varying the temperature from 400 to 600 °C and after its metabolic manipulation through heterotrophic growth. The bio-oil yield increased up to a maximum of 57.9% at 450 °C.

Jena and Das [11] studied pyrolysis of *Spirulina platensis* at high temperatures (500 °C, 60 min, heating rate of 7 °C min^{-1}) and low temperatures (350 °C, 60 min, heating rate of 3.5 °C min^{-1}), comparing it with hydrothermal liquefaction of the same microalgae at 350 °C and 60 min. The bio-oil yield was higher for pyrolysis at 500 °C (29%) than at 350 °C (23%), being both yields significantly lower than that from hydrothermal liquefaction (41%). Also, the bio-oil produced through pyrolysis appeared to have lower energy content and stability properties than that produced by liquefaction. Moreover, pyrolysis was found to produce less energy than it consumed, mainly due to the high energy penalty of the drying process of the wet microalgae, which contained around 80% of moisture. Despite the advantage of processing the whole microalgal biomass, this high energy cost is a significant hindrance that may avoid a complete development of this technology.

2.1.3. *Gasification*

Conventional gasification consists in the conversion of a carbonaceous material through a partial oxidation of biomass at elevated temperatures (higher than 700 °C), carried out in practice at atmospheric pressures. Very little data is available with regard to the use of microalgae as feedstock for gasification processes. Again, the high moisture content of the feedstock seems to be an insurmountable barrier for the development of this technology.

Hirano et al. [17] gasified at temperatures from 850 to 1000 °C a microalgae paste containing a mass fraction of 21% of *Spirulina* and analyzed the exit gas in view of producing methanol from it. The produced gas was mainly composed out of H_2, CO_2 and CH_4. The maximum theoretical yield of methanol was 64% at 1000 °C, higher than the values from woody biomass, which they report to be in the vicinity of 45–50%.

2.2. *Wet processes*

2.2.1. *Hydrothermal carbonization*

Hydrothermal carbonization (HTC) aims to the production of char products. This process uses the mildest temperature and pressure conditions of all the wet conversion routes: temperatures around 200 °C and pressures lower than 2 MPa. The process is carried out in water and is exothermic and spontaneous, leading to two product streams: an insoluble charry product and an aqueous phase.

Heilmann et al. [18] investigated HTC of several microalgae strains (*Chlamydomonas reinhardtii*, *Synechocystis* sp., *Aphanizomenon flos-aquae*, *Spirulina* spp., *Chlorella* spp. and *Dunaliella salina*) at different conditions (190–210 °C, 0.5–3 h, 5–25% mass fraction of solids load), and found that mild conditions, such as 200 °C and a residence time of 30 min were sufficient to obtain an adequate degree of carbonization (yield of 45.7%, with a carbon recovery in the algal charry product of 62%).

The low value of the solid product obtained has resulted in few studies available about microalgal HTC. However, this process has lately been suggested as an intermediate step towards the production of biodiesel from lipid-rich microalgae [19,20]. During HTC, the lipids naturally present in microalgae undergo hydrolysis and form fatty acids that appear to be adsorbed at a high percentage (more than 90% of their mass) on the surface of the charry product obtained. These fatty acids could subsequently be subjected to a solid–liquid extraction (i.e. with ethanol) that could ideally simultaneously transesterify the fatty acids to produce biodiesel, while the aqueous phase, with a high content of nutrients, could be recycled to grow more algae. This solid–liquid extraction would also avoid the typical emulsification and phase separation problems from the liquid–liquid extractions used for traditional biodiesel production methods.

2.2.2. *Hydrothermal liquefaction*

Hydrothermal liquefaction (HTL) is a biomass-to-liquid conversion route carried out in water at medium temperatures (280–370 °C) and high pressures (10–25 MPa) that has a liquid biocrude as main product, as well as gaseous, aqueous and solid phases by-products [21]. The biocrude produced, with an energy value close to fossil petroleum [11], is not directly suited as transportation fuel, but it is expected to be a suitable renewable feedstock for co-refining in existing fossil-based refineries.

In the last years, the number of papers related to microalgal HTL has significantly increased, which indicates a raising interest for this conversion technology. Temperatures covering the whole HTL range, as well as reaction times varying from 5 to 120 min have been investigated for various kinds of strains (*Botryococcus braunii*, *Spirulina platensis*, *Chlorella vulgaris*, *Nannochloropsis* sp. or *Desmodesmus* sp., among others) [2,22–25]. Maximum biocrude yields in the vicinity of 50–60% have been reported, and the use of several homogeneous and heterogeneous catalysts has been already analyzed [24,26,27], though in a very early stage of development. Although initially this process was assessed for lipid-rich strains [22], some recent studies suggest that other microalgae with lower lipid content but higher growth rates might constitute a suitable feedstock for this technology [2]. The hydrothermal liquefaction process will be the focus of the present review, and will be analyzed in detail in the following sections.

2.2.3. *Hydrothermal gasification*

This route is also usually referred as supercritical water gasification (SCWG). In this process, water is in a supercritical state (above 374 °C and 22.1 MPa) and acts as both solvent and reactant. Typical conditions for this process are 400–700 °C and 25–30 MPa. Several authors have dealt with SCWG of microalgae, testing different strains (e.g. *Phaeodactylum tricornutum*, *Spirulina platensis*, *Nannochloropsis* sp. or *Chlorella vulgaris*). Also various process conditions are reported in the literature: temperatures ranging from 360 to 700 °C, reaction times from 1 to 90 min and algae mass fraction loadings from 2.5 to 20% have been tested [28–32]. These experiments have been carried out in volumes varying from quartz capillaries (approximately 0.5 mL) to small batch reactors (30 mL), also testing different catalysts that lead to a strong increase of the gas yields. Ru catalysts gave the highest gasification efficiencies, ranging from 75% to total gasification within several studies [28,30].

The dry gas composition of uncatalyzed SCWG was mainly constituted by CO_2, CO, CH_4, H_2, and some C_2–C_3 compounds. High temperatures, low algae concentrations, and long residence times enhance the gas yields [30–32]. Even a kinetic model was proposed [32], based on a set of reaction pathways comprising two types of intermediates: one that reacts quickly to form gases, and other that reacts more slowly and requires therefore longer reaction times.

Despite the advantages associated to this technology (high efficiency, use of direct wet biomass and possibility of recycling the minerals dissolved in the process water to the microalgae culture medium) SCWG is associated with high energy costs (due to the high temperatures required) and capital costs (the materials need to be able to withstand the harsh conditions from this process). Moreover, the value of the gas obtained does not seem to be competitive with its production costs.

3. Hydrothermal liquefaction of microalgae

3.1. *Process description and status of technology*

The conventional approach for making biofuels from microalgae has been to subject high-lipid strains to subsequently energy-intensive thermal drying, solvent extraction and transesterification to produce biodiesel. These steps are costly and require the use of organic solvents unhealthy to both humans and the environment. Moreover, lipid-rich strains are usually slow-growing microorganisms (hence lower cultivation productivity), and the lipid mass fraction generally varies between 20 and 50% [33], thus leaving a large fraction of the microalgae biomass unconverted.

In order to make microalgae an economically viable alternative for biofuel production, the revenues from all their fractions (and not only the lipids) need to be maximized. In this context, hydrothermal liquefaction appears to be a promising medium temperature and high pressure thermochemical conversion technique that processes the whole microalgae biomass in order to produce a liquid energy carrier (biocrude oil). Just like for SCWG, the high water content of microalgae makes them a good raw material for hydrothermal liquefaction in subcritical water. The higher value of the primary product and the lower energy requirements compared to other technologies turn liquefaction to be a very promising, if not the most promising conversion technology for microalgae conversion. The process conditions reported in literature usually range from 250 to 375 °C, 10–20 MPa and microalgal mass fractions of 5–20% in the slurry feed, which can be achieved while consuming only 12% of the energy needed for their complete dewatering [34].

Only simple process schemes can be traced in the literature. A completely defined process description, including estimated mass and energy balances, is still lacking. García Alba et al. [2] provided an approach to a whole biorefinery process scheme, under the name of ABC (Algae Biorefinery Concept), which is presented in Fig. 2. It consists of four main steps: growth, harvesting, fractionation and hydrothermal processing.

There are various systems available for the growing step [6,8]. At a lab scale, mainly small fermentors or photobioreactors are used, while for large scale applications, either open ponds or closed systems are common technologies. To attain axenic cultures, photobioreactors appear as the best technology. However, in our envisaged process scheme, axenity does not seem to be a “must” and therefore open ponds appear as a suitable option to make this technology economically feasible, due to their current lower cost. Once cultured, the microalgae need to be harvested, to concentrate them to the required loading rate for the intended thermochemical process. Although several techniques are available, the best route seems to be to carry out a stepwise harvesting: first flocculation, followed by either flotation or sedimentation, and subsequently centrifugation or filtration [35].

For the thermochemical step, either the whole algae or the residual fraction after extraction of value-added products (e.g. lipids, saccharides or protein material) can be used. If value-added products other than biofuels are to be obtained, a fractionation step is required prior to the hydrothermal liquefaction. The fractionation is generally considered to be a combination of a pretreatment step with an extraction. Mechanical cell disruption, ultrasound or PEF (pulse electric field) are typical pretreatment (fraction-disclosing) methods. The whole biomass or the remainings after the fractionation would thereafter undergo the thermochemical conversion step, thus producing a biocrude oil, plus gas, aqueous and solid phases. Ideally, the aqueous phase would contain a high portion of the nutrients from the microalgae cultivation method and could be recycled to the growth area, while the other fractions may undergo upgrading processes to maximize their utilization potential. This combination of fractionation and a thermochemical conversion step has been suggested as a route for obtaining proteins as a value-added co-product, while reducing the nitrogen content of the biocrude obtained by subsequent thermochemical conversion of the remaining biomass [2]. However, this fractionation step would come at the expense of a reduction of the overall biocrude oil yield.

In the past decades, several pilot- and demo-scale projects for hydrothermal liquefaction of terrestrial biomass were

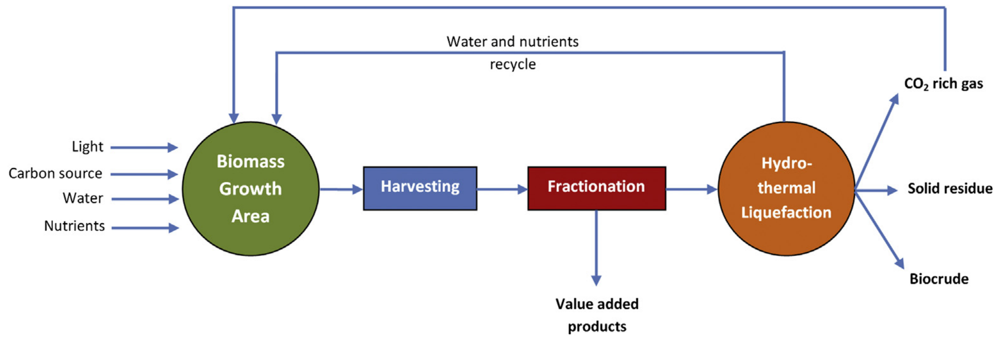


Fig. 2 – Conceptual scheme for an algae biorefinery (adapted from García Alba et al. [2]).

started, but none of them succeeded. However, the recent raise in the fuel prices has triggered again the interest in this technology. HTL technology started developing during the seventies mostly through short-term projects for terrestrial biomass that failed to achieve a demonstration scale, due to a combination of technological and economic problems that made them unfeasible, underlining its difficulties. No large scale attempt using microalgae as feedstock has been carried out yet. The main reason for the failure of the earlier projects is that they were started when still many questions about the process remained (and still remain) open. The main problems encountered were:

- When working with biomass, it is difficult to ensure a feed with constant properties and composition over time. Biomass impurities and inhomogeneities (e.g. particle size) and their handling are critical issues, as they can precipitate and cause plugging of the reactor or poison the catalyst active sites.
- The mass fraction of solids in the feed for neutral economic operation needs to be around 5%, as lower concentrations would result in an energy-negative mode of operation due to heat losses and pumping required to circulate the water.
- Mixtures of >15% biomass mass fraction in water are difficult to pump into the reactor at the high pressures required. These rheology-related problems are likely to be less important when using microalgae as biomass feedstock, especially at low concentrations, due to the microscopic size of the cells.
- Heterogeneous catalysts in continuous operation are prone to fouling and undergo deactivation.
- Resistant materials (e.g. stainless steel) are needed to withstand the corrosive environment caused by subcritical water.
- Reaction pathways and kinetics are still unclear, as well as the function and type of catalysts adequate for this process.

Several articles have reviewed the state of the art of HTL technology for terrestrial biomass [21,36–39]. Already in the early 1940s some liquefaction experiments were developed with terrestrial biomass, but it was after the oil crisis of 1973 when this technology gained relevance as an alternative for biofuel production that could reduce the dependency on fossil petroleum. By this time, the pilot plant of Albany (Oregon) was erected. It was first run by researchers from the Pittsburgh Energy Research Center (PERC) and then from the Lawrence Berkeley Laboratories (LBL) from Berkeley (California). In both cases, the basic problem was injecting the biomass in the reactor at high concentrations and at a rate without affecting the product distribution and without mechanical problems. During the operation, and especially during the PERC period, innumerable mechanical problems were encountered, mainly related to an inadequate equipment design and material selection. The research was halted by the US Department of Energy at the beginning of the 1980s, when the petroleum price decreased.

New attempts from the LBL, the University of Arizona and the University of Saskatchewan also failed to achieve a commercially attractive process. In the 1980s, the Shell Laboratory in Amsterdam developed the Hydrothermal Upgrading (HTU®) process. Stopped in 1988 due to unfavorable economic conditions, the project was resumed in 1997 by the Dutch company Biofuel. Again, the introduction of the feed in the reactor was a critical issue, as well as the heating of the reactants and the treating of the effluent water. A pilot plant has been erected and operated for 100 kg h^{-1}, with the aim of producing sufficient technical and economic data to study the feasibility of a commercial demonstration plant at a scale of 25,000 Mg y^{-1} of dry biomass, which so far has not been erected.

Several companies have attempted to develop and commercialize this technology. The CatLiq® Process, from the Danish company SCF Technologies, is one of the few active endeavors in this field. It is a continuous process that converts organic waste into bio-oil at subcritical conditions (350 °C and 25 MPa), using homogeneous (K_2CO_3) and heterogeneous (zirconia) catalysts [40].

3.2. *Chemistry of the process*

Hot compressed subcritical water has some characteristics very different from water at standard conditions [21]: a lower permittivity, varying from 78 F m^{-1} at 25 °C and 0.1 MPa to 14.07 F m^{-1} at 350 °C and 20 MPa, which causes a rise in solubility of hydrophobic organic compounds; the hydrogen bonds are fewer and weaker; and the ionic product of water is two orders of magnitude higher (10^{-12} compared to 10^{-14} at 25 °C), promoting a high availability of H^+ and OH^- for acid- and base-catalyzed reactions such as hydrolysis. These properties change at supercritical conditions, where radical reactions dominate, enhancing gasification reactions.

The mechanism of hydrothermal liquefaction has not yet been elucidated. The influence of various factors, like temperature, heterogeneous or homogeneous catalyst dosage, holding time or ash content remains still unclear. It is generally stated in the literature that in hot compressed liquid water, close to the critical point (374 °C and 22.1 MPa), competition between two reactions takes place: hydrolysis and repolymerization [2,41]. The first one has more importance at the early stages of the process, when the microalgae are decomposed and depolymerized to small compounds. These compounds may be highly reactive, thus polymerizing and forming biocrude, gas and solid compounds [24,42,43]. When increasing the reaction time or the temperature, repolymerization, condensation and decomposition of the components from the different phases may occur. This may lead to an increase of the solid and gas yields and a reduction of the biocrude oil yield [44–46]. This is consistent with the fact that biocrude viscosity decreases with increasing holding times [47]. A schematic representation of the HTL pathways is shown in Fig. 3.

Biller and Ross [24] studied HTL of different model components that represented the lipid, protein and carbohydrate fraction of microalgae, and compared the results with the hydrothermal liquefaction of certain microalgae strains. They proposed that the conversion efficiencies toward biocrude formation followed the trend: lipids (55–80%) – proteins (11–18%) – carbohydrates (6–15%). This contrasts with the results provided by other authors [48,49], who obtained high

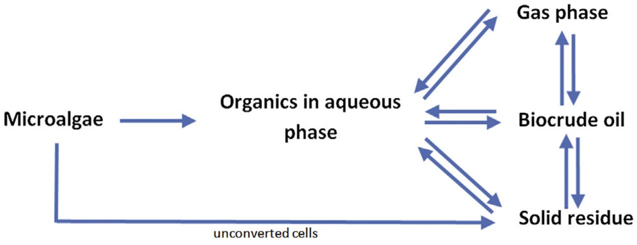


Fig. 3 – Scheme of HTL kinetic pathways.

yields by using strains with very low lipid content. Biller and Ross [24] even created an equation to estimate the biocrude oil yield as an additive relation between the mass fraction of each algae fraction in the cell multiplied by their respective individual yields, obtained from the liquefaction of model compounds.

$$\begin{aligned}\text{Biocrude oil yield}(\%) = {} & \text{Protein content}(\%)\cdot\text{Protein yield}(\%)\\ & +\text{Carbohydrate content}(\%)\\ & \cdot\text{Carbohydrate yield}(\%)+\text{Lipid content}(\%)\\ & \cdot\text{Lipid yield}(\%) \qquad (1)\end{aligned}$$

This equation was fitting well only for two of the strains tested in their study (*Nannochloropsis occulata* and *Chlorella vulgaris*). By applying the equation to other studies under the same reaction conditions big deviations can be found between the predicted yields and the reported ones, as shown in Table 1.

It seems then that each microalgae fraction does not behave independently during the HTL process and its contribution to the overall yield cannot be considered as a linear addition, but an interaction among them by means of several cross-linked reactions (e.g. Maillard reactions between carbohydrates and proteins, or condensations between degradation products from lipids and proteins [3]).

Although the conversion mechanism remains quite unclear, some authors have attempted to suggest some conversion routes for the different fractions during HTL of terrestrial biomass [21,39,50] and microalgae [24]. Torri et al. [3] reported that at mild conditions (lower than 250 °C) the main constituents of the biocrude oil were derived mostly from the liquefaction of lipids and algaenans, while more severe conditions (300–375 °C) promoted the conversion of carbohydrates and proteins, the latter increasing the nitrogen content of the biocrude oil.

3.2.1. *Conversion of lipids*

Microalgal lipids are usually in the form of triacylglycerols (TAGs), mainly with an aliphatic character. They consist of a glycerol backbone bounded to three fatty acids. Glycerol is one of the products obtained from the hydrolysis of TAGs. By means of HTL, glycerol is converted into methanol, acetaldehyde, propionaldehyde, acrolein, allyl alcohol, ethanol and formaldehyde, and gas products, mainly CO, CO_2 and H_2 [51]. Fatty acids have a higher thermal stability, but they can be converted into long-chain hydrocarbons.

3.2.2. *Conversion of proteins*

Proteins are polymers of amino acids linked by peptide bonds, which quickly hydrolyze at HTL conditions. The yield of amino acids is low though, as they undergo further decarboxylation to produce carbonic acid and amines, and deamination to produce ammonia and organic acids. The products from these reactions may then repolymerize to long chain hydrocarbons and aromatic ring type structures like phenols or nitrogen heterocyclics such as indole or pyrrole [21,24,39]. They are the main source of nitrogen in the resulting biocrude oil.

3.2.3. *Conversion of carbohydrates*

The direct degradation products from carbohydrates do not convert to biocrude oil. The main liquefaction mechanism is believed to be the carbohydrates breaking down to polar water-soluble organics, such as organic acids (i.e. formic, acetic, lactic), aldehydes, benzenes and alcohols, all carrying a substantial amount of oxygen [21,24,39]. The aldehyde- and benzene-type structures may further produce larger hydrocarbons, which are then part of the biocrude fraction.

3.2.4. *Conversion of algaenans*

Not much information is available about the conversion of algaenans. These insoluble macropolymers of hydrocarbons, more resistant to chemical treatments than other microalgae fractions, appear to yield directly to hydrocarbons with variable length (alkanes and alkenes) and alkyl-aromatics [3]. Their resistance makes it also difficult to analyze their behavior and no relevant data is available about the chemical pathways of this fraction in HTL processes.

3.3. **HTL products**

3.3.1. *Biocrude oil*

Biocrude oil is the dark and viscous, energy-dense liquid produced from hydrothermal degradation of the microalgae constituents (lipids, proteins, carbohydrates and algaenans).

Table 1 – Microalgae biochemical composition (mass fraction of the microalgae feedstock, dry basis), and comparison of the biocrude oil yields (mass fraction of the dry input biomass) via HTL at 350 °C predicted by Equation (1) versus the reported yields from two literature studies.

Author	Strain	Lipids	Proteins	Carbohydrates	Predicted oil yield	Reported oil yield
Brown et al. [23]	*Nannochloropsis* sp.	28	52	12	27.7 ± 8.3	43 ± 2
Jena et al. [25]	*Spirulina platensis*	11.2	49.2	31.2	18.0 ± 6.4	39.9

It has an energy content of 70–95% of that of petroleum fuel oil [23] and it is similar in nature to a heavy crude [52]. A typical elemental composition (in mass fraction) of the biocrude oil produced from *Desmodesmus* sp. at 375 °C and 5 min of reaction time was provided by García Alba et al. [2]: N 6.3%, C 74.5%, H 8.6% and O 10.5%. It had a significantly lower oxygen content than the original microalgae cells (33.9%), and its higher heating value (HHV) was 35.4 MJ kg^{-1}.

The physical and chemical properties of biocrude oil appear to be strongly dependent on feedstock and production conditions. It is a complex mixture of a great number of compounds with a wide range in molecular weight. A partial characterization of HTL oils can be obtained by gas chromatography analysis, but most of the heavy compounds do not get eluted into the column and remain uncharacterized. Torri et al. [3] identified three large classes of compounds: lipid derivatives (mainly fatty acids and sterols), protein derivatives (peptides, and/or cyclic peptides, amongst others) and heavy insoluble materials (algaenan derivatives and asphaltenes), the relative amount of each being affected by the experimental conditions, especially the temperature. The main problem related to this biocrude oil is its high content of N, usually in the vicinity of 5–7%, leading to high NO_x emissions upon combustion. The sulfur content should also not be overlooked, as it is normally in the vicinity of 0.5–1% [11,23].

3.3.2. *Aqueous phase*

The handling of the aqueous phase obtained after HTL is critical, due to its high content in organic matter and nutrients. Its main constituents are PO_4^{3-}, NH_4^+, CH3COO$^-$, besides metallic cations such as K^+, Na^+ or Mg^{2+} [52,53]. Microalgae can assimilate nitrogen from a wide variety of sources, either organic or inorganic, and some microalgae strains have demonstrated their capability to grow, albeit with slower rates, in cultures with the nitrogen source being recovered from the water fraction remaining after the liquefaction process [54,55]. The high content of organics in the aqueous phase may also be interesting as carbon source for heterotrophic strains.

Another interesting handling alternative, apart from direct recycling to the culture medium, is the gasification of this phase at supercritical conditions to obtain a hydrogen-rich fuel gas. After this process, however, most of the nutrients would ideally still remain dissolved in the outcoming aqueous phase, allowing their recycling to the growth step.

3.3.3. *Gas phase*

The gaseous fraction presents a yield of approximately 20% of the original organics present in the microalgae feedstock [2,11,23]. CO_2 is the main gas product of HTL, followed by H_2. At 350 °C, Brown et al. [23] provided the following gas molar composition: 662 mmol mol^{-1} CO_2 and 297 mmol mol^{-1} H_2, the rest of the gas fraction constituted of low amounts of CH_4, N_2, C_2H_4 and C_2H_6. Several authors report the concentration of CO_2 to decline once the critical point of water is surpassed, while the concentration of small hydrocarbons (CH_4 and C_2) increases. The low amounts of CO reported in the literature might indicate that the oxygen removal in HTL mainly occurs by decarboxylation, instead of decarbonylation [2], or that the CO formed readily reacts and forms CO_2 and H_2 by the water–gas shift reaction [56]. This second route might be enhanced if the microalgae slurry is accompanied by salts from the growth medium.

The CO_2 produced could be recirculated to the microalgae production system, while the H_2 may be used for further hydrotreating of the produced biocrude. Hence, this fraction is attractive from a biorefinery integration point of view.

3.3.4. *Solid residues*

Microalgal HTL also yields a solid residue having a high content of ash, and very little hydrogen, sulfur and nitrogen. Almost all the authors report solid yields lower than 10% [43,47,52]. An elemental composition of the solid residue from HTL of *Spirulina platensis* was provided by Jena et al. [25], at 350 °C and 60 min of reaction time:11.82% C, 1.81% H, 1.41% N and 0.61% S. These solid residues are likely to contain some nutrients, which makes them attractive as a soil amendment. Moreover, they may be used as feedstock for subsequent thermochemical processes, such as pyrolysis or gasification, that would produce other energy carriers, while the remaining ashes could be recycled as nutrients for microalgal growth. However, no detailed study is available on the composition and applications of this fraction following HTL.

4. Status of the research in hydrothermal liquefaction of microalgae

4.1. *Strain selection for HTL*

Various important factors need to be taken into account to select a microalgae strain for biofuel production, such as the growth rate, the resistance to environmental change, the nutrient preference, the ease of separation from water, the ease of processing or the possibility of obtaining high value products, other than biofuels [6,57]. Amaro et al. [57] and Malcata [58] suggested that for large-scale production purposes, marine microalgae are more suitable, as salinity prevents extensive pollution in the culture medium, while eliminating the need of freshwater. However, it needs to be pointed out that for HTL purposes a high purity of the culture medium is not a must.

As already mentioned, algae researches have aimed mainly to find strains with a high lipid content. The two parameters mostly investigated were biomass productivity and lipid productivity. Much importance was given to the second parameter, as the main interest was the production of biodiesel, and therefore a high fraction of lipids was required to make that process economically viable. In contrast, for hydrothermal conversion, the overall biomass productivity appears to be of much more importance, as this conversion technology processes the entire microalgae. Biomass productivity and lipid content of the strains are generally inversely related parameters, as the synthesis of lipids has a higher metabolic cost than proteins or carbohydrates. A thorough study of thirty strains carried out by Rodolfi et al. [59] showed that the best biomass producers were four marine strains: *Porphyridium cruentum* and three *Tetraselmis* strains, all of them with a low lipid content.

The main advantages and drawbacks of the strains that have been mostly used in the HTL experiments reported in the literature are:

- *Nannochloropsis* sp.: it appears to have the best combination of biomass productivity and lipid content [59,60]. This strain presents a rapid and robust growth, a high lipid content (up to 40% in some cases), and presence of algaenans [4]. Its main drawback relays on the small spherical shape of the cells, with a thick cell wall that makes their conversion more difficult [61].
- *Dunaliella tertiolecta*: a deep knowledge is already available for its mass culture for β-carotene production, a bioactive compound in food industry [49]. It can achieve high growth rates without the need of sterile conditions, which is advantageous for a further scale-up process [62].
- *Botryococcus braunii*: Metzger and Largeau [63] and Yoo et al. [64] highlighted its considerable combined production of lipids and hydrocarbons, which makes it a very interesting source for biofuel production. It has a high tolerance towards several chemical pollutants, which makes it capable of growing using industrial flue gases, and the hydrocarbons bulk is located in the outer walls of the algae, which facilitates its extraction [65]. Despite these advantages, *Botryococcus braunii* is known as a slow growing organism with a low resistance to biological contamination, which hinders its mass cultivation [57].
- *Spirulina*: this cyanobacteria has been part of the human diet for many decades, and it is extensively cultivated around the world. It tends to form aggregates, which simplifies the harvesting. Its high protein content may be a problem for biocrude production due to its high nitrogen content.
- *Chlorella vulgaris*: this green algae is a fast growing freshwater microorganism, with a high growth rate and ability to accumulate a high concentration of lipids under stress. Flue gases can be used as carbon source, due to its ability to grow under high CO_2 concentrations.

In the context of this review, the literature available for several strains was studied in terms of their use as source for hydrothermal conversion, and the information gathered is shown in Table 2. Special attention was paid to the biomass productivity, with the aim to find strains with a robust and fast growth, in view of maximizing the production of feedstock for HTL processes. The ease of cultivation, the chemical composition and the presence of algaenans were also important parameters taken into account, mainly in accordance to the information reported elsewhere [66]. The signs (+) and (−) indicate if a species is good or bad in terms of each of the parameters shown in columns, while the value 0 indicates that the species is neutral with regard to that parameter.

Considering the data provided by Table 2, *Porphyridium cruentum*, *Phaeodactylum tricornutum*, *Scenedesmus* sp. and *Tetraselmis* sp. appear to be promising strains due to their high biomass productivity. Strains like *Nannochloropsis* sp., *Dunaliella tertiolecta* or *Scenedesmus* sp. seem interesting due to the presence of algaenans. *Chlorella vulgaris* is often mentioned as an interesting strain for waste water treatment, an important feature that would significantly contribute to make HTL a sustainable and economically viable technology. These mentioned species are reported to have a robust growth, which is interesting for mass culture purposes. On the other hand, *Neochloris oleoabundans* and *Botryococcus braunii* do not appear to be suitable for HTL, due to their slow growth rate, although they have been mentioned in the literature as a good feedstock for biofuel production due to their composition [63,70].

4.2. *Biocrude oil yield definition*

The analysis of the biocrude yield becomes a crucial parameter in the overall process, as the biofuel production is the main objective. Among the different papers available to date, a huge variability in terms of the biocrude yields can be found, even for the same strain at identical operating conditions. This may partially be caused by the various ways of

Table 2 – Strain selection for HTL and typical biochemical composition of several species (expressed as mass fraction of the microalgae feedstock, dry basis).

Habitat	Species	Biomass productivity[a]	Ease of cultivation	Lipids (%)	Carbohydrates (%)	Proteins (%)	Algaenans[f]
Marine	*Tetraselmis suecica*	+	+	20[b]	20[b]	40–50[b]	−
	Porphyridium purpureum	+	+	9–14[c]	40–57[c]	28–39[c]	−
	Nannochloropsis gaditana	0	+	18[b]	36[b]	29[b]	+
	Phaeodactylum tricornutum	+	+	18[b]	26[b]	36[a,b]	n/a
	Dunaliella tertiolecta	0	+	22[d]	20[d]	32[d]	+
Freshwater	*Scenedesmus* sp.	+	+	12–14[c]	10–17[c]	50–56[c]	+
	Chlorella vulgaris	0	+	14–22[c]	12–17[c]	51–58[c]	−
	Neochloris oleoabundans	−	n/a	19[b]	8[b]	54[b]	n/a
	Botryococcus braunii	−	−	21[c,e]	n/a	n/a	−

n/a: not available.
a Chen et al. [67].
b AquaFuels [66].
c Becker [68].
d Zou et al. [41].
e Yoo et al. [64].
f Versteegh and Blokker [69].

calculating the yields that the different authors apply. García Alba et al. [2], Dote et al. [22], Biller and Ross [24], Biller et al. [26], Yang et al. [43], Minowa et al. [47] and Vardon et al. [71] calculated the oil yield as the ratio of the weight of biocrude obtained from liquefaction to the dry weight of the organic matter in algal cells, without considering neither the catalyst used nor the ash present in the microalgae. Matsui et al. [72] extracted the reaction mixture with tetrahydrofuran (THF). The THF-soluble fraction was further diluted with hexane and separated into hexane-insoluble and hexane-soluble fractions, being the hexane-soluble fraction noted as oil. The yield was obtained using the percentage of converted microalgae, and subtracting the fractions corresponding to the hexane insolubles and the gas phase. Ross et al. [52] introduced the mass of catalysts in the yield calculation. One last possibility takes into account the whole mass from the raw material, including the ash [23,25,27,41,48,49,73]. Table 3 shows the maximum biocrude oil yields reported in the literature, even though their comparison is difficult, given that the authors calculated them on non-equivalent basis. The equations used by each author are also included in Table 3. It also shows the experimental conditions used (temperature, reaction time) as well as the catalyst load (expressed as mass fraction of the dry microalgae feedstock or as concentration in the microalgae slurry, depending on the author). The high nitrogen content of the biocrude oil reported by each author is also highlighted, showing that it is one of the most difficult challenges for making biofuels from microalgae a reliable commercial technology.

Considering the catalyst or the ash content in the yield calculation might significantly alter the result: in some of the studies considered in this review, the catalyst mass fraction used was as high as 50% of the algal biomass liquefied; and the ash content might play a catalytic role in the HTL process [24]. In our opinion, Equation (a) from Table 3 would bring the most meaningful results, as only the compounds that can actually be liquefied and converted into biocrude oil are considered.

4.3. *Methods and conditions used*

To the present date only lab scale experiments for microalgae HTL can be found in the literature. They are usually carried at temperatures of 300–375 °C and at residence times of 60 min. Both heterogeneous and homogeneous catalysts have been tested. The reactor volumes used are usually below 100 mL, and there is also a large variation in the agitation type: unstirred, stirred and shaken reactors have been used, although no specific comparison between them is available. In this section, the different methods reported in the literature will be commented, as well as the parameters and the effect of their variation.

4.3.1. *Microalgal physical state and load*

There are large differences in the literature regarding the initial physical state of the microalgae for HTL experiments:

Table 3 – Literature studies available on microalgae HTL – experimental conditions, maximum biocrude oil yield reported and its nitrogen content (mass fraction).

Reference	Strain	T (°C)	Holding time (min)	Catalyst load	Yield (%)	N (%)
Dote et al. [22]	*Botryococcus braunii*	300	60	Na_2CO_3 (5%)	64[a]	0.7
Minowa et al. [47]	*Dunaliella tertiolecta*	300	5	No cat.	43.8[a]	6.7
Yang et al. [43]	*Microcystis viridis*	340	30	Na_2CO_3 (5%)	33[a]	7.1
Biller and Ross [24]	*Chlorella vulgaris*	350	60	No cat.	~36[a]	5.9
	Nannochloropsis occulata	350	60	No cat.	~35[a]	4.1
	Porphyridium cruentum	350	60	Na_2CO_3 (1 mol L^{-1})	27.1[a]	3.2
	Spirulina	350	60	No cat.	~29[a]	7.0
García Alba et al. [2]	*Desmodesmus* sp.	375	5	No cat.	49[a]	6.3
Vardon et al. [71]	*Spirulina*	300	30	No cat.	32.6[a]	6.5
Matsui et al. [72]	*Spirulina*	350	60	No cat.	61[b]	6.8
Ross et al. [52]	*Chlorella vulgaris*	350	60	Na_2CO_3 (1 mol L^{-1})	27.3[c]	4.6
	Spirulina	350	60	Na_2CO_3 (1 mol L^{-1})	20[c]	4.9
Biller et al. [26]	*Nannochloropsis occulata*	350	60	No cat.	34.3[c]	4.1
	Chlorella vulgaris	350	60	Pt/Al_2O_3 (20%)	38.9[c]	5.6
Zou et al. [49]	*Dunaliella tertiolecta* (cake)	360	50	Na_2CO_3 (5%)	25.8[d]	3.7
Zou et al. [41]	*Dunaliella tertiolecta*	360	30	No cat.	36.9[d]	5.0
Brown et al. [23]	*Nannochloropsis* sp.	350	60	No cat.	43[d]	3.9
Duan and Savage [27]	*Nannochloropsis* sp.	350	60	Pd/C (50% daf)	~57[d]	3.9
Jena et al. [25]	*Spirulina platensis*	350	60	No cat.	39.9[d]	6.,3
Valdez et al. [73]	*Nannochloropsis* sp.	350	60	No cat.	39[d]	4.4
Yu et al. [48]	*Chlorella pyrenoidosa*	280	120	No cat.	39.4[d]	n/a

daf: dry, ash free.

a $\text{Oil mass fraction}(\%) = (\text{Weight of oil}(g)/\text{Weight of organic matter in the algal cells}(\text{daf})(g))\cdot 100.$

b $\begin{array}{c}\text{Conversion mass fraction}(\%) = (\text{Feed}(g) - \text{THF}_{\text{insolubles}}(g) - \text{Catalyst residue}(g)/\text{Feed}(g))\cdot 100\\ \text{Oil mass fraction}(\%) = \text{Conversion}(\%) - \text{Hexane insolubles}(\%) - \text{Gas}(\%)\end{array}.$

c $\text{Oil mass fraction}_{\text{organic basis}}(\%) = (\text{Biocrude mass}(g)/\text{Algae mass}(g)\cdot(100 - H_2O(\%) - \text{Ash}(\%)/100) + \text{Organic content of the catalyst}(g))\cdot 100.$

d $\text{Oil mass fraction}(\%) = (\text{Weight of biocrude oil}(g)/\text{Weight of raw material}(g))\cdot 100.$

the use of pulverized dried samples [41,49], freeze dried pulver cells mixed with deionized water [24–26,48,52] and intact microalgae cells as received from the culture medium [23,27,43,47,71,73] has been reported. By using pulverized samples, the extractability of some constituents might be altered, thus affecting the yield and composition of the biocrude oil. In our opinion, data provided by experiments with microalgae paste as received from the culture medium is more meaningful, as it represents a more realistic scenario for the implementation of this technology, and it also allows a more accurate study of the fate of nutrients and the overall feasibility of the process.

Experiments with a microalgae mass fraction in the feed ranging from 4 to 50% in the initial mixture in the reactor can be found in the literature, with contradictory findings. This indicates that the optimum ratio microalgae:water remains still unclear. A high microalgal load can result in a reduction of the amount of water available to stabilize and dissolve the microalgae cells [41,74], but it reduces the energy required for heating the water. Jena et al. [25] reported that increasing the biomass mass fraction in the feed from 10 to 20% led to an increase of more than 20% in the biocrude oil yield and a reduction in the organics dissolved in the aqueous fraction, although opposite findings are reported elsewhere [2].

4.3.2. *Temperature*

Temperature appears to be a critical parameter in the HTL process. The typical operation temperatures reported in the literature are in the range of 300–375 °C. At subcritical conditions, an increase in the reaction temperature appears to increase the biocrude yield [2,25,41,43,49,52], as shown in Fig. 4. Also the biocrude properties are strongly dependent on the reaction temperature [2,47]: increasing the temperature leads to a decrease in the oxygen content and a consequent higher HHV [25,47]. The nitrogen content also increases, likely due to the promotion of protein degradation at high temperatures. By surpassing the critical point of water, the biocrude yield starts to decline. Supercritical water enhances radical-induced cracking of biocrude molecules to form lighter, more volatile compounds that form gas, which is consistent with the higher gas yields at supercritical conditions present in many studies.

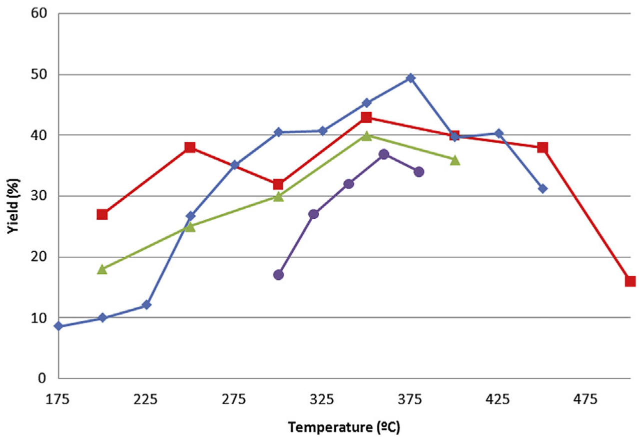


Fig. 4 – Influence of the temperature in the biocrude oil yields reported by Brown et al. [23], –■–; Zou et al. [41], –●–; García Alba et al. [2], –◆– and Jena et al. [25], –▲–.

At the subcritical range of temperatures, the yield of organics in the aqueous phase appears to decrease with an increase in temperature, indicating that higher temperatures promoted the conversion of intermediate water-soluble products. On the other hand, the gas yield increases with higher temperatures [2], as well as the concentrations of CH_4 and C_2 hydrocarbons [2,23,43]. The solid yield decreases with higher temperatures, being this decrease very sharp at low temperatures (200–300 °C) [2].

4.3.3. *Holding time*

The holding time is defined as the period during which the maximum temperature is maintained for HTL, not accounting for the heating and cooling periods. The effect of this parameter is closely linked to the temperature: to attain a high biocrude oil yield, higher temperatures require lower holding times. In any case, the holding time most frequently applied in the literature studies is 60 min. There may be an opportunity to optimize the reaction time and temperature in order to minimize the cost of the process. As an example, Yu et al. [48] obtained a biocrude oil yield of 39.4% at 280 °C and 120 min, while García Alba et al. [2] attained 49.4% with only 5 min, but at 375 °C. However, for continuous operation these values may vary significantly and more research and kinetic data is needed in this area. The use of a continuous-flow reactor for hydrothermal processing of microalgae at subcritical conditions has been recently reported by Elliot et al. [75]. In that study, feeding rates of 1.0–1.5 L h^{-1} were used for mass fractions of microalgae in the feed slurry of 8.0–25.0% of microalgae. However, the focus of that study was the gas production and the recoverability of nutrients in the aqueous phase, and no relevant information is provided about the production of biocrude oil.

4.3.4. *Heating rate*

The heating rate as a parameter is usually not mentioned in the literature. Only few studies give information about the heating rate [2,23,24,26,27]. The effect of this parameter on the biocrude yield remains still unclear. The results for *Nannochloropsis occulata* and *Chlorella vulgaris* [24,26] show that no effect can be noticed by increasing the heating rate from 10 to 25 °C min^{-1}. Other available studies use higher heating rates, in the vicinity of 100 °C min^{-1} [23,27]. These studies are also within the ones reporting the highest biocrude oil yields, which could indicate a positive effect from higher heating rates in increasing the biocrude oil formation. However, more information needs to be generated regarding this topic.

4.3.5. *Catalysts*

Homogeneous catalysts: They are often mentioned in the literature for having a positive effect on microalgae HTL [43,49,52], mainly in the form of alkali salts like Na_2CO_3 or KOH. They appear to improve the liquefaction yields and decrease the solid residues. These catalysts cause a rise in the pH, which inhibits the dehydratation of biomass molecules and promotes decarboxylation. It is also believed that they enhance the water-gas shift reaction, favoring the formation of H_2 and CO_2 [56]. The H_2 gas may act as reducing agent, thus increasing the heating value of the oil [21].

Na_2CO_3 is commonly considered as a good catalyst for microalgae HTL, based on previous experiences with lignocellulosic biomass. Other homogeneous catalysts, such as formic or acetic acid, have also been tested, but they seem to lead to biocrude oils with a higher oxygen content [52]. Moreover, there are concerns about the role that they play, as they appear to be consumed during the process, so they might be considered reagents rather than catalysts.

Heterogeneous catalysts: Several heterogeneous catalysts have been reported in the literature: Pd, Pt or Ru supported on C; CoMo, Ni, Pt, Ni/SiO_2 supported on Al_2O_3; and zeolite. They can present several drawbacks, such as sintering, dissolution, poisoning or intraparticle diffusion limitations. Duan and Savage [27] tested six different heterogeneous catalysts, changing the headspace environment used during the reaction (inert with He or reducing with H_2). The addition of catalysts promoted the production of biocrude when an inert environment was used. Although the biocrude oil yields were affected by the presence or absence of catalysts, the increase in yield was similar for every catalyst tested. When using a reducing environment with H_2, the trend changed: the yield was higher for the uncatalyzed process. The elemental composition and heating values were largely insensitive to the catalyst used, and even to its presence. Contrary to these results, Biller et al. [26] also tested heterogeneous catalysts in HTL (Co/Mo, Ni/Al and Pt/Al) and concluded that their effect was clearly noticeable in the higher heating value and the level of de-oxygenation, more than in the biocrude oil yields. Heterogeneous catalysts attained also significant de-nitrogenation levels. With the available data, and given the fact that they can be easily recovered from the reaction mixture (compared to homogeneous catalysts), heterogeneous catalysts seem to be a promising option to improve the HTL process towards an economically feasible energy production alternative. However, the knowledge generated on this topic to the present is scarce and contradictory and a more systematic approach to test both types of catalysts is needed.

4.3.6. *Product separation*

Following HTL, the different fractions obtained need to be separated. Almost all the experiments reported so far in the literature use an organic solvent (dichloromethane, chloroform, acetone, THF or hexane) to separate the biocrude oil from the aqueous and solid phases, but no extensive attention is paid to the effect of this solvent on the biocrude yield and quality. Table 4 shows the different product separations described in the literature for HTL experiments. Only experiments in water without added catalysts are reported here, to avoid the disturbance in the biocrude yield of any other parameter. The solvent added is also indicated and, when known, its volume per mass of dry weight of algae.

As it can be seen in Table 4, no clear trend can be found regarding the influence of the solvent per dry mass of algae loaded to the reactor. However, Valdez et al. [73] tested several organic solvents for HTL product separation, and found that the solubility of the biocrude oil molecules was varying within them. Both biocrude oil yields and composition were affected by the solvent used. For the same strains (*Nannochloropsis* sp.) and same process conditions, yields were varying from 39% with decane and hexadecane to 30% with dichloromethane. This indicates that the solvent type and amount used for separating the various HTL products might have a strong influence in the biocrude oil collection, although not enough data is presently available.

An organic solvent increases the overall cost of the process, and needs to be further evaporated, which could lead to the lighter, volatile biocrude oil molecules evaporating along with the solvent, thus reducing the yield. This separation technique is needed to recover the low volumes of product obtained from the small-scale experiments present in the literature. However, if a continuous operation is to be pursued, the separation should ideally be carried out by gravity, as the biocrude and aqueous phases appear to spontaneously form separate phases, especially at temperatures close below to the critical temperature of water.

Table 4 – Product separation with different solvents reported in the literature for microalgae HTL without added catalysts

Author	Solvent	$V_{solvent}:dw_{algae}$ ($mL \cdot g^{-1}$)	Oil yield (%)	Strain
Minowa et al.[47]	DCM	n/a	43.8	*Dunaliella tertiolecta*
Matsui et al. [72]	THF+DCM	n/a	61.0	*Spirulina*
Yang et al. [43]	Chloroform	n/a	28	*Microcystis viridis*
Zou et al. [49]	Chloroform	n/a	22	*Dunaliella tertiolecta* (cake)
Brown et al.[23]	DCM+Water	50+50	43.0	*Nannochloropsis* sp.
Biller and Ross[24]	DCM+Water	17+17	36	*Chlorella vulgaris*
Duan and Savage[27]	DCM	64	36	*Nannochloropsis* sp.
Biller et al.[26]	DCM+Water	17+17	35.8	*Chlorella vulgaris*
Jena et al. [25]	Acetone	n/a	39.9	*Spirulina platensis*
García Alba et al. [2]	DCM	47	49.4	*Desmodesmus* sp.
Yu et al. [48]	Toluene	n/a	39.4	*Chlorella pyrenoidosa*
Vardon et al.[71]	DCM	n/a	32.6	*Spirulina*
Valdez et al. [73]	Decane	2	39	*Nannochloropsis* sp.

4.4. *Mass, energy and nutrients balances*

Although the yields from each fraction are strongly dependent on the process parameters, some general trends can be described regarding the mass balances. The main fractions are the biocrude oil and the dissolved organics in the aqueous phase, while the amounts of gas and solid residues produced are lower. It is important to note that in several papers not all product-phase yields are directly measured. Some of them are calculated by difference, especially the yields referring to the aqueous phase, which can lead to overestimations in the yield of this phase.

Regarding the energy balance of HTL, there is no clear agreement in the literature about its profitability. There is even no agreement in the way of defining the energy yield. Most of the studies consider two parameters to assess the energy balance: the energy recovery and the energy consumption ratio. The energy recovery is the ratio of energy present in the products to the energy of the original microalgae. However, some authors consider ‘produced energy’ only that stored in the biocrude [2,11,24,52], while others include the energy content of the gas product [23]. It needs to be remarked that no study considers the energy stored in the solid product, although it might have some applications following HTL. Regarding the energy consumption ratio, there is clear agreement in calculating it as the ratio of the energy required for the HTL to the energy stored in the biocrude produced [11,24,47]. Biller and Ross [24] suggested that HTL resulted in energy losses, and that only a good economic balance would make HTL an economically feasible technology. In contrast, Jena and Das [11] and Minowa et al. [47] reported HTL to be a net energy producer. It should also be pointed out that all these reported energy balances overlook some important energy inputs to the process, such as the algae growing step or the pumping.

The heating value of microalgal feedstock is usually around 20 MJ kg^{-1}, while the values from biocrude are closer to that of petroleum oil (43 MJ kg^{-1}) [23]. Fig. 5 shows a dry mass and atomic balance (expressed as mass fraction) adapted from the hydrothermal liquefaction of *Spirulina platensis* for 60 min at 350 °C [25]. It also includes the heating values for the biocrude oil and solid residue phases. In the referred study no data about the heating value of the gas is mentioned, but Brown et al. [23] report it to be in the vicinity of 4 MJ kg^{-1} from HTL experiments with *Nannochloropsis* sp. at the same conditions as the study used as basis for Fig. 5.

Xu et al. [34] presented the results of the energy balance analysis for a dry and a wet route for the production of biofuels from microalgae. The dry route consisted in drying the microalgae in order to extract the lipids for biodiesel production, while the remaining oil cake was pyrolyzed to produce biogas and pyrolysis oil. In the wet route, the microalgae underwent a wet extraction to separate the lipids for green diesel production, while the remaining aqueous phase was processed via supercritical water gasification (SCWG) to produce hydrogen and other gases. The study concluded that both routes could achieve a positive energy balance. This encourages further analysis for the HTL process, which would consume less energy than SCWG, besides processing the whole wet microalgae biomass.

The fate of nutrients is a critical issue, as they account for a high input cost during the growing step. Ideally, the aqueous phase resulting from HTL would contain a high percentage of the nutrients from the culture medium, and could be recycled again to be re-used to grow more microalgae biomass. Some authors have analyzed the distribution of nitrogen within the HTL products [2,11], concluding that in fact a high percentage of the N ends up in the aqueous phase produced during the thermochemical conversion (from 40 to 75% of nitrogen recovered). Different catalysts lead to large variations in the nitrogen recovery [52], which needs to be taken into account when selecting catalysts for the process. Not only the biocrude oil yield should be optimized, but also the possibility of recycling nutrients and thus reducing the costs for the growing step. Few studies are available regarding the possibility of reusing nutrients for subsequent microalgae growth after HTL [53,54,76], but the results are promising and indicate that microalgae growth and HTL can be carried out with integrated nutrient recycling.

The mass, energy and nutrients balances need to be considered together for a proper assessment of the feasibility of HTL technology. The optimal conditions to maximize the various balances seem to be different, requiring a compromise solution. As an example, Biller et al. [26] reported that

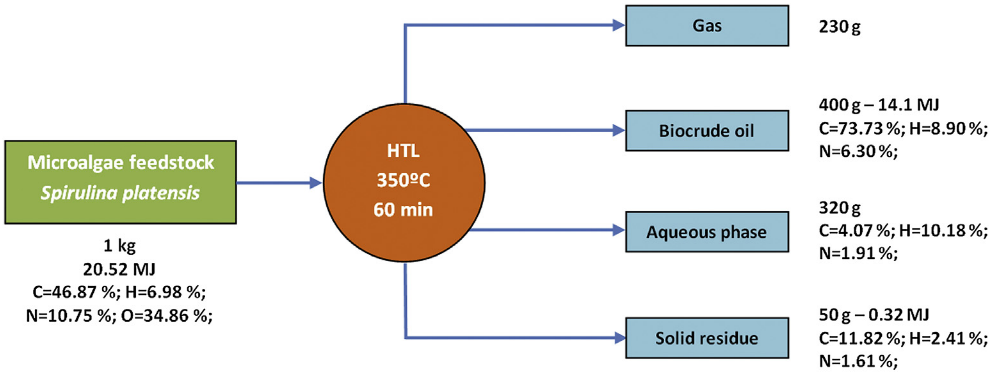


Fig. 5 – Typical mass and atomic balance (mass fraction, dry basis) for hydrothermal liquefaction (adapted from Jena et al. [25]).

decreasing the liquefaction temperature from 350 to 300 °C reduced the energy consumption by 22% and only by 3% the biocrude oil yield.

5. Conclusions and directions for future research

Hydrothermal liquefaction of microalgae appears to be a very promising technology for biofuel production, but still in a very early stage of development. While this technology has been already used for other kinds of biomass, a deep knowledge on its fundamentals and applicability to microalgal feedstock still lacks. A literature research was carried out to bring together what has already been published. Significant variations were found in the way the different researchers carry out the experiments and analyze the results, which makes it difficult to compare the data. However, bringing together all the work done to the present allowed to reach some conclusions. In order to maximize the biocrude oil yield, temperatures close to the critical point of water seem the most adequate, with holding times in the vicinity of 5 min. Longer times, closer to 60 min, may even lead to decomposition of biocrude. Most of the authors report a beneficial effect from the use of catalysts in the biocrude yield and its heating value. With regard to homogeneous catalysts, alkalis appear to be a better option than organic acids: they lead to higher heating values and higher biocrude oil yields. However, heterogeneous catalysts appear to be worth a bigger effort in their development, due to the promising results delivered by some first experiments, in terms of biocrude yield and properties. Especially interesting is their positive effect in the de-nitrogenation.

On the other hand, many issues remain still unclear and require further research. All the data presently available for microalgae HTL is obtained from experiments carried out in small batch mini-autoclaves, stirred, unstirred or shaken. No comparative study is available and tests with continuous setups are still lacking. The product separation following the thermochemical conversion is also a critical issue than may significantly affect the biocrude oil yield. Deeper analyses need to be carried out to assess the possibility of gravity separation. The heating rate might have some influence in the recovery of some microalgae compounds, although no study on this field is currently available. Experiments should be carried out trying to reproduce the real conditions as close as possible. For this reason, the use of microalgae paste as obtained from the culture medium would lead to more meaningful results, in view of assessing the suitability of strains for HTL and the fate of nutrients during the whole process chain, from culture to liquefaction.

New experiments with different microalgae strains are needed to develop a deeper understanding of the influence of the cell structure and composition on the process yields. Compared to technologies like solvent extraction for biofuel production, HTL does not require high-lipid strains. As it allows to process the whole microalgae biomass, fast and robust growing organisms seem more suitable, thus improving the overall economic balance of the process. The assessments of the energy balances of similar technologies indicate that microalgae HTL could become in the next years an economically feasible technology for biofuel production, but still much research needs to be devoted to this field to completely understand this conversion process.

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